

Addendum Report to “Investigation of Pre-washed Mixed Bagged Salad following an Outbreak of *Escherichia coli* O157:H7 in San Diego and Orange County”

Date: April 2004 – October 2005

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Background

In 2002, a Washington State outbreak implicated romaine lettuce grown in Salinas, California. In 2003, two outbreaks, one implicating spinach and the other implicating a lettuce mixture (romaine and iceberg), were also traced to the Salinas Valley. Chinn Ranch 3, in Salinas, California was identified in all three of these outbreaks as one possible source for the spinach, iceberg lettuce, or romaine. Because no commonalities among the three outbreaks were identified other than Chinn Ranch 3 as a possible grower and supplier, California Department of Health Services Food and Drug Branch (CDHS-FDB) staff developed and implemented a study to further examine Chinn Ranch 3.

Summary

Portions of Chinn Ranch 3 periodically flood from overflow water from the Santa Rita Creek that borders the property. In addition, composted manure was used on portions of this ranch. From June 2004 through December 2005, over 800 environmental samples were collected in the Salinas Valley and Chinn Ranch 3 area and tested for *E. coli* O157:H7, fecal and total coliforms, thermo-tolerant coliforms, and/or non-pathogenic *E. coli*. One Santa Rita Creek (SRC) sediment sample tested by United States Department of Agriculture – Agriculture Research Service (USDA-ARS) laboratory personnel was positive for *E. coli* O157:H7. The PFGE pattern of this isolate did not match any isolates in the FDB Microbial Disease Laboratory (MDL) database. The isolate was also unique in the PulseNet database. All other samples tested were negative for *E. coli* O157:H7. The Western Institute for Food Safety and Security (WIFSS) tested feces from 15 Canada geese and 14 other animals found in the Salinas Valley and Castroville, California. All samples tested negative for *E. coli* O157:H7. Possible animal contamination sources were identified in the SRC environment upstream from Chinn Ranch 3. Further environmental study is being conducted in collaboration with the USDA-ARS.

Description of Chinn Ranch 3

The approximately 200-acre Chinn Ranch 3 is located on the northwest side of Salinas, California and is divided into 20 growing fields (Attachment 1). Comgro, a custom grower leases this ranch. SRC, which is a county-maintained agricultural ditch, borders the north side of Chinn Ranch 3. The east and west sides of the ranch are bordered by farmland and the south side is mostly farmland with a few houses and farm structures. Another agricultural ditch borders the east side of Chinn Ranch 3-8 and drains into the SRC. Chinn Ranch 3 is bowl-shaped and sits upon a drained lake and is therefore highly susceptible to flooding from rainfall.

History of Chinn Ranch 3

During the “Investigation of Pre-washed Mixed Bagged Salad following an Outbreak of *Escherichia coli* O157:H7 in San Diego and Orange County” in 2003, Mr. George Fontes, Comgro partner, stated that the SRC had a history of flooding parts of Chinn Ranch 3. Depending on the amount of rainfall, one to six fields (fields 3, 5, 6, 7, 8, and 11) of Chinn Ranch 3 can be flooded or partially flooded during the rainy season. Mr. Fontes stated that another cause of flooding on Chinn Ranch 3 had been the failure of a pump located on SRC adjacent to Chinn Ranch 3-11 (Attachment 2). This pump is maintained and operated by the Monterey County Water Resource Agency (MCWRA). Sections of the ranch become completely submerged during minor rainfall (Attachment 3). Mr. Fontes told CDHS-FDB staff that he contacted Monterey County officials in May 2003 when Chinn Ranch 3-11 was flooded due to a malfunction of the pump.

A Monterey County Health Department (MCHD) laboratory report showed fecal coliform levels of 3,000 MPN and total coliform levels >24,182 MPN for a 100 ml water sample collected May 16, 2003 at a time when parts of Chinn Ranch 3 were flooded (Attachment 4). This sample was collected 1000 feet upstream of the pump on SRC. These test results were an indication that the SRC was exceeding the objectives of the Central Coast Water Board numeric objectives for fecal coliform. The Water Quality Control Plan for the Central Coast Region 3 (Basin Plan) considers a single sample containing greater than 400 MPN per 100 ml fecal coliform to be a violation of an existing water quality objective for REC I waters (Attachment 5). The SRC is classified as a REC I water source under the Basin Plan and is therefore required to meet these water quality standards.

Comgro also owns a composting facility approximately four miles from Chinn Ranch 3 where raw grape pomace, cow and chicken manure, and green waste are composted. Comgro makes different types of compost depending on the needs of individual farmers who purchase the material and Comgro also uses this compost on their leased fields including Chinn Ranch 3. Upon completion of the Pat and Oscar investigative report and reviewing the data and information from the three outbreak investigations, CDHS-FDB staff identified two facts that warranted further investigations, 1) a positive *E. coli* O157:H7 Canada geese feces sample collected from the Sella Ranch, Castroville, California and 2) Chinn Ranch 3 was a possible source for produce involved in three consecutive *E. coli* O157:H7 lettuce and/or spinach outbreaks. CDHS-FDB staff initiated discussions with concerned parties about these geographical areas and investigation of possible *E. coli* O157:H7 contamination sources. Personnel from Monterey County agencies, CDHS-FDB, United States Food and Drug Administration (FDA), Regional Water Quality Control Board (RWQCB), University of California, Davis Cooperative Extension (UC Davis), WIFSS, and USDA-ARS met in April

2004 and decided to further study the Chinn Ranch 3, SRC, and Canada geese habitat areas in the Salinas Valley. The Environmental Sampling Project 2004 (ESP) was developed as a result of this and other meetings (Attachment 6).

The Environmental Sampling Project (ESP) 2004

The ESP outlined a multi-agency approach to investigate potential sources of *E. coli* O157:H7 on and around Chinn Ranch 3 and to collect Canada geese feces in the Salinas Valley. The ESP outlined collection techniques and testing methods for environmental samples. Chinn Ranch 3, Comgro Compost, and the SRC were the main areas of focus for the ESP. The samples included well and recycled water, animal feces, romaine, iceberg lettuce, spinach, compost, compost water run-off, SRC and Tembladero Creek ditch sediment and water, irrigation water runoff, and drag swabs. WIFSS collected Canada geese feces from areas identified by USDA wildlife specialist in Monterey County where birds were observed feeding or inhabiting. Mr. Fontes volunteered to plant Chinn Ranch 3-8 as a test plot for the ESP. Mr. Fontes cultivated three, approximately one-acre areas (A, B, & C), on field 8 of Chinn Ranch 3 (Chinn Ranch 3-8). Each acre was planted with four rows each of broccoli, spinach, romaine, and iceberg lettuce. Each of the three test areas was visually divided in half thus producing the sampling areas 1A, 2A, 1B, 2B, 1C, and 2C (Attachment 7). Global Positioning System (GPS) points were recorded for the sampling areas. Area A was amended with grape pomace compost (Lot # 218), area B with composted chicken and cow manure (Lot #231), and area C did not have compost applied. The other approximately 12 acres of Chinn Ranch 3-8 were left uncultivated. Comgro did not harvest these crops, but tilled all produce upon maturity.

CDHS-FDB staff met and discussed possible SRC contamination sources with Monterey County Department of Environmental Health (MCDEH) staff several times in 2004. MCDEH staff were provided photographs of animals with access to the SRC, trash and debris in the creek or on the creek banks, and given the location of a goat farm and a horse ranch that drained into SRC. Further study of the SRC and the Salinas Valley is being planned for 2005.

Environmental Investigations

Santa Rita Creek Environment (SRC) and Surrounding Areas

The SRC is a collection source for agriculture and urban run off water. A pond on the northeast side of the junction of Herbert and San Juan Grade roads in Salinas, California serves as its source. SRC flows west through agriculture and urban areas, drains into Tembladero Slough, which drains into the Reclamation Ditch, which eventually empties into the Pacific Ocean near Moss Landing, California (Attachment 8). The creek travels through farm, residential, and public lands before it reaches the north side of Chinn Ranch 3 (Attachment 9).

A goat ranch located at 745 Boronda Road, Salinas, California bordered the west side of an agricultural drainage ditch (Attachment 10). This ditch flowed into another ditch that (approximately one-half mile downstream) bordered the east side of Chinn Ranch 3-8 before it drained into the SRC (Attachment 11). On October 13, 2004, CDHS-FDB staff observed approximately 30 goats corralled on a small hill above this agricultural drainage ditch. During the rainy season, runoff from the goat area drains into the previously described

drainage ditch. Horses and cows were also kept on this property.

On the south side of the SRC, a few hundred yards upstream from the goat farm was a horse ranch with approximately 12 horses (Attachment 12). The horses had complete access to the creek as observed by CDHS-FDB staff on numerous occasions. CDHS-FDB staff observed horses and cows in other places along the Santa Rita Creek upstream from the horse ranch to the pond off of Herbert Road (Attachment 13).

Environmental Samples

Laboratory Samples and Methods

Over 800 environmental samples were collected and analyzed on or near Chinn Ranch 3-8 for *E. coli* O157:H7, generic *E. coli* and/or indicator bacteria. Environmental samples consisted of drag swabs, SRC sediment, plants, and water, irrigation run off water, feces, soil, romaine, iceberg lettuce, spinach, well and recycled water, and compost. Most of the samples were analyzed for the presence of *E. coli* O157:H7. UC Davis Laboratory did not analyze all samples for this pathogen, but rather tested for indicator bacteria and conducted further analyses for *E. coli* O157:H7 if warranted by the preliminary test results.

The following table displays the type of samples analyzed by individual laboratories:

Laboratory	Drag swabs	Feces	Well and recycled water, Templadero Slough water	SRC sediment, plants and/or water	Compost	Romaine, iceberg, and/or spinach	Soil	Irrigation runoff water
USDA-ARS		X		X	X	X	X	
UC Davis				X	X	X	X	X
FDB-Richmond						X		
FDA-Alameda	X							
MCDEH			X	X				
WIFSS		X						

FDA-Alameda, MCDEH, WIFSS, and USDA-ARS laboratories followed a modified Bacteriological Analytical Manual (BAM) method for *E. coli* O157:H7 sample analyses. Approximate numbers for each type of sample collected and analyzed by these laboratories (not including UC Davis analyses) are as follows: SRC sediment (80), drag swabs (55), well and recycled water (14), SRC water (8), Templadero Slough water (9), feces (29), SRC plants (16), iceberg, romaine, and spinach (66), SRC bank soil (3), and Comgro compost (25). The FDB-Richmond Laboratory staff used Coli-lert to determine the concentrations of total coliform and generic *E. coli* on iceberg lettuce, romaine, and spinach samples. FDB Laboratory staff chemically analyzed all positive generic *E. coli* test results for *E. coli* O157:H7. Of the environmental samples tested, all but one, previously mentioned SRC sediment sample were negative for *E. coli* O157:H7.

The UC Davis Cooperative Extension Laboratory staff collected and analyzed approximately 515 environmental samples. Approximate numbers for each type of sample collected and analyzed are as follows:

	Number of Samples Tested	
	Indicator Bacteria†	<i>E. coli</i> O157:H7
Compost	66	0
Lettuce Chinn Ranch 3-8	60	0
SRC Sediment	15	15
Soil	246	214
SRC & Runoff Water	128	54
TOTAL	515	283

†Presumptive *E. coli* and thermo-tolerant coliforms

SRC = Santa Rita Creek

UC Davis staff did not use the BAM method for sample analysis, but used their previously developed laboratory protocol methods. All samples were analyzed for baseline indicator bacterial populations suggestive of fecal contamination. A media (CHROMagar® ECC) specifically designed for detection and enumeration of presumptive *E. coli* and thermo-tolerant coliforms was used. A subset of samples also was analyzed for the presence of *E. coli* O157:H7 by enrichment (Universal Preenrichment Broth) and selective/differential agar plating using a comparison of a new enrichment procedure for shiga toxin-producing *E. coli*. Enrichment broths and individual suspected colonies were tested for the *stx1* and *stx2* genes by PCR. Thus, if a test result indicated the possible presence of *E. coli* O157:H7 further tests were conducted to verify the presence or absence of this pathogen. All samples tested for the presence of *E. coli* O157:H7 were negative. Attachment 14 shows data tables for the different laboratory samples. Attachment 15 contains individual reports from laboratory personnel responsible for testing ESP samples.

Collection Methods

All samples were placed on ice upon collection and delivered to the appropriate laboratory within 24 hours of collection. Sterile Whirl-Pak bags, drag swabs, scoops, gloves, and containers were used for all samples.

The drag swab was a sterile four ply, three-inch by three-inch cotton gauze swab (Solar-Cult from Solar Biologics, Inc., pre-moistened with double strength skim milk) attached to a sterile cord. Two different methods were used to introduce the swab to the soil. One technique was to simply pull the swab by the attached string over the area of interest. The other method was removal of the string from the swab (aseptically) and attach the swab to a sterile clamp on the end of a stainless steel rod (the rod method). The rod was then used to prod the ground or drag over the ground with the attached swab. Using aseptic technique, the drag swab was pulled on the ground to the side of the sampler or prodded using the rod method. Two gloved samplers walked approximately 60 yards into different sections of a test plot, each collecting soil samples onto a drag swab. The two swabs were placed into the same Whirl-Pak bag. The procedure, using two additional swabs, was repeated for the same section and added to the Whirl-Pak bag with the first two swabs, thus producing one composite sample. All samplers wore sterile gloves for the collections. Collection of drag swabs was discontinued after October's collection because the Chinn Ranch 3 fields were no longer cultivated and would remain inactive until the next growing season.

Water samples were collected in sterile bottles, placed on ice, and transferred to the laboratories. Using sterile scoops (or gloved hand) samples of compost, creek plants, iceberg

lettuce, spinach, romaine, creek sediment, and feces were collected and placed into Whirl-Pak bags. Attachment 16 shows photographs for sample collections and collection areas.

Recommendations

1. Agricultural ditches, such as the SRC, frequently carry run-off water from grazing lands, dairies, or small backyard farms. Livestock are known to carry and shed human pathogens such as *E. coli* O157:H7. Thus, these waterways have the potential to be contaminated with human pathogens, especially following heavy rainfall. Therefore, ready-to-eat crops should not be planted on fields known to be subject to flooding by these agricultural ditches.
2. Ready-to-eat crops that have been flooded with water from these agricultural ditches, creeks, or similar tributaries should not sold be for human or animal consumption. Testing of ready-to-eat crops exposed to flood waters cannot provide adequate assurance of the safety of this product. The U.S. Food and Drug Administration has issued similar guidance following floods in Pennsylvania and Louisiana (Attachment 17).
3. Canada geese can harbor *E. coli* O157:H7 and other human pathogens. Growers should evaluate the feasibility of control measures for large resident populations of Canada geese or other wildlife known to carry *E. coli* O157:H7. USDA Wildlife Services offers several techniques to manage Canada geese on fields.
4. Agricultural ditches, such as the SRC, may transport viable pathogens such as *E. coli* O157:H7. Therefore, measures to prevent ditches from overflowing should be implemented when possible. These measures may include removing silt build-up to allow maximum flow and maintaining all pumping stations to reduce malfunction events.
5. Local agencies should review and enforce local codes and ordinances in regards to the dumping of trash and household wastes into agricultural ditches, such as the SRC, that are adjacent to ready to eat crops. Local agencies should also review and enforce local codes and ordinances regarding animal manure and leaking septic tanks entering tributaries that are known to flood fields containing ready to eat crops.
6. A comprehensive study of the Salinas Valley and its agricultural ditches is necessary to better understand the prevalence, survival, and sources of *E. coli* O157:H7 in this environment. Federal agencies such as USDA-Cooperative State Research, Education, and Extension Service (CSREES) should incorporate funding to more fully assess these environments.

Conclusions

1. Chinn Ranch 3 was the only grower common to all three *E. coli* O157:H7 investigations.
2. The specific source of *E. coli* O157:H7 contamination for these lettuce-associated outbreaks has not been determined.
3. The SRC bordering Chinn Ranch 3 contained *E. coli* O157:H7 in July 2005, which did not match, via PFGE analysis, any outbreak strains recorded by FDB-MDL or CDC.
4. *E. coli* O157:H7 was not found on Chinn Ranch 3, produce grown on Chinn Ranch 3 for the ESP, or in compost sampled from Comgro Compost, Salinas California during this study.

Attachments

1. Map: Chinn Ranch 3 (1 page)
2. Photo: SRC pump station (1 page)
3. Photos: Chinn Ranch 3 (1 page)
4. May 19, 2003 Monterey County Health Department laboratory results (1 page)
5. Central Coast Region 3 Basin Plan objectives for REC I waters (2 pages)
6. The Environmental Sampling Project 2004 (26 pages)
7. Map and Photos of ESP Chinn Ranch 3 test plot areas (4 pages)
8. Map of Santa Rita Creek (1 page)
9. Photos: Santa Rita Creek Environment (6 pages)
10. Photos: Goat farm (2 pages)
11. Photo: Irrigation ditch bordering Chinn Ranch 3-8 (1 page)
12. Photos: Horse ranch off of Boronda Road, Salinas, CA (13 pages)
13. Photos: Santa Rita Creek Environment and Farm Animals (1 page)
14. Individual laboratory data: A.) UC, Davis (68 pages) B.) FDA, Alameda (6 pages) C.) WIFSS (2 pages) D.) FDB, Microbial Disease Laboratory (4 pages) E.) USDA-ARS (19 pages) F.) Monterey County Health Department (4 pages)
15. Individual laboratory reports: A.) UC, Davis (9 pages) B.) FDA, Alameda (2 pages) C.) WIFSS (4 pages) D.) FDB, Microbial Disease Laboratory (2 pages) E.) USDA-ARS (2 pages) F.) Monterey County Health Department (2 pages)
16. Photos: ESP sample collections (1 page)
17. FDA Center For Food Safety And Applied Nutrition (CFSAN) "Katrina Recovery Update" Bulletin 1
FDA Memorandum: CFSAN Position On Human Food Crops Affected By Flood Waters (5 pages)